Reference
Veal, M. 2019. *Magnitude of the Residue of Spiromesifen in Cotton Pollinator Matrices after Application with Spiromesifen SC 240 (240 g/L) in North America. Final Report.* Unpublished study performed by Bayer CropScience LP, Research Triangle park, North Carolina, and sponsored by Bayer CropScience LP, St. Louis, Missouri. Study ID: EBBS0027. Study completed May 7, 2019.

1. STUDY INFORMATION

Chemical:	Spiromesifen	**PC Code**	024875
Test Material #1	OBERON® 2 SC	**Purity**	23.3%
Study Type:	Non-Guideline field residue study on cotton to establish spiromesifen and metabolite levels in nectar (floral and extrafloral), pollen and leaves following two foliar applications.		
Sponsor:	Bayer CropScience LP, 800 N. Lindbergh Blvd, S. Louis, MO 63167	**Performing Laboratories:**	(**Trial 01):** Carolina Ag-Research Service, Inc., P.O.Box 132, Elko, SC 29826
Report Number:	EBBS0027		(**Trial 02):** Excel Research Services, Inc., 4545 N. Brawley Ave., Suite 105, Fresno, CA 93722
Study Completion Date:	May 7, 2019		(**Trial 03):** South Texas Ag Research – RGV, Inc., 891 W. Fox, Raymondville, TX 78580
Experiment Start/End Date:	June 5, 2018 to January 25, 2019		(**Trial 04):** Turner Ag Research, 2760 Duncan Rd., Yuba City, CA 95993
Study Location:	4 Field Trials: Elko, South Carolina (Trial 1; BS001-18ZA); Madera, California (Trial 2; BS003-18ZA); Raymondville, Texas (Trial 3; BS007-18ZA); Meridian, California (Trial 4; BS009-18ZA)		**(Analytical Analyses):** Bayer CropScience LP, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709
GLP Status:	GLP-compliant; 40 CFR Part 160		

2. REVIEWER INFORMATION

Primary Reviewer: Daniel Hunt, M.S., Environmental Scientist, CSS-Dynamac

Signature: Dan Hunt **Date:** 07/23/2019

Primary Reviewer: Cameron Douglass, Ph.D. Biologist, USEPA/OPP/EFED/ERBIV

Signature: Cameron Douglass 2020.01.27 14:48:32 -05'00' **Date:** 01/27/2020

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

3. EXECUTIVE SUMMARY

This study was designed to measure the magnitude of residues of spiromesifen and spiromesifen-enol in cotton pollen, nectar (floral and extrafloral), and leaves. Four separate trials were conducted, at one location in South Carolina, two locations in California, and one location in Texas. One plot at each trial location received two foliar treatments of Oberon® 2 SC at a nominal application rate of 0.25 lb ai/A. The first application was made to cotton plants between BBCH 61 (beginning of flowering) and BBCH 63 (30% of flowers open), and the second application was made 7 days later. Cotton pollen and nectar (extrafloral and floral) samples were collected from each trial for residue analysis on three occasions beginning at 0 Days After the Last Application (DALA) and ending at 6 DALA. Leaf samples were only collected at 0 DALA.

A summary of the key findings is as follows:

1. Two foliar applications of Oberon® 2 SC to cotton plants at BBCH 61-63 at nominal application rates of 0.25-0.26 lb ai/A yielded detectable residues of spiromesifen in pollen and nectar throughout the 0-6 DALA study period at all trial sites, and leaves at 0 DALA.
2. In cotton matrices, spiromesifen residues were greatest in leaves (mean maximums of 40.61-54.53 mg/kg for each trial site), followed by extrafloral nectar (mean maximums of 0.36-12.17 mg/kg for each trial site), pollen (mean maximums of 0.8-6.96 mg/kg for each trial site), and floral nectar (mean maximums of 0.05-0.12 mg/kg for each trial site). The parent material accounted for the majority of total recovered residues in leaves, nectar, and pollen, while spiromesifen-enol residues were greatest in extrafloral nectar, followed by leaves, pollen, and floral nectar (mean maximums ranged from 0.02-2.81 mg/kg for each trial site).

Analyte	Matrix	Maximum Measured Concentration (mg/kg)	Study Site	Maximum Average Concentration (mg/kg)	Study Site
Spiromesifen	Extrafloral Nectar	12.17*	Madera, CA	12.17*	Madera, CA
	Floral Nectar	0.25	Meridian, CA	0.12	Meridian, CA
	Pollen	8.24	Elko, SC	6.96	Meridian, CA
	Leaves	61.89	Meridian, CA	54.53	Meridian, CA
Spiromesifen -enol	Extrafloral Nectar	2.81*	Madera, CA	2.81*	Madera, CA
	Floral Nectar	0.02	Elko, SC	0.02	Elko, SC
	Pollen	0.64	Meridian, CA	0.42*	Madera, CA
	Leaves	1.8	Meridian, CA	1.41	Meridian, CA
Total	Extrafloral Nectar	13.15*	Madera, CA	13.15*	Madera, CA
	Floral Nectar	0.25	Meridian, CA	0.13	Meridian, CA
	Pollen	8.45	Elko, SC	7.24	Meridian, CA
	Leaves	63.69	Meridian, CA	55.95	Meridian, CA

* Pooled sample.

3. Trends in spiromesifen and total spiromesifen residue concentrations following two foliar applications declined in pollen samples from 0 DALA to 6 DALA at all trial sites excluding Trial 2 (Madera, California), where maximum pooled residues were observed at 6 DALA. Spiromesifen and total spiromesifen residues declined in extrafloral nectar samples from Trials 1-3 following maximum mean concentrations detected at 0-3 DALA and from floral nectar samples following 0 DALA (insufficient

extrafloral nectar samples were collected at Trial 4 to determine a trend).

4. DT50 values of spiromesifen, spiromesifen-enol, and total spiromesifen residues could not be calculated in nectar, pollen, or leaves at all trial sites due to an insufficient number of sampling intervals.

4. STUDY VALIDITY

Guideline Followed:	Non-guideline study (protocol was reviewed by EPA/PMRA/CDPR)
Guideline Deviations:	N/A
Other Deviations:	N/A
Classification:	ACCEPTABLE
Rationale:	No major deviations were identified in this study that would affect the scientific integrity of this study.
Reparability:	N/A

5. MATERIALS AND METHODS

Test Material Characterization			
Test item:	OBERON® 2 SC	**CAS #:**	283594-90-1
Description:	Suspension concentrate	**Purity:**	23.30%
Lot No./Batch No.	NTR7HX1495	**Density:**	Not Reported
Material Source:	Not reported	**Cert. #**	218GS7645
Material Receipt Date:	Not reported	**Analysis Date:**	March 20, 2018
Expiration Date:	3/20/2020	**Solubility:**	Not Reported
Storage of Test Mat'l:	Ambient (40-84°F)	**Sample Storage:**	Not Reported

5A. STUDY DESIGN

This study was conducted to quantify the magnitude and decline of residues of spiromesifen and spiromesifen-enol in cotton matrices following two foliar applications of Oberon® 2 SC at 0.25-0.26 lb ai/A, which represents the seasonal maximum number of foliar applications permitted according to the label. The first test application was made to cotton plants between BBCH 61 (beginning of flowering) and BBCH 63 (30% of flowers open), and the second application was made 7 days later. One test plot (*ca*. 300 x 288 ft, Trial 1; *ca*. 225 x 75 ft, Trial 2; *ca*. 450 x 152 ft, Trial 3; and *ca*. 1500 x 40 ft, Trial 4) was established at each trial site, and divided into three subplots. Samples of cotton pollen and nectar (extrafloral and floral) were collected at 0, 2-3, and 6 DALA at all sites, with leaves collected only at 0 DALA, and analyzed for residue concentrations. Spiromesifen-enol (4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one) residues were also quantified.

5B. APPLICATION TIMING AND RATES

Two foliar applications were made to cotton plants at each test site in June-October, 2018. Test applications were made at 0.25-0.26 lb ai/A, between BBCH 61 (beginning of flowering) and BBCH 63 (30% of flowers open). All applications were made using ground-based equipment. The study author did not report supporting details on the nozzles used. Application volumes ranged from 13 to 19 GPA. Information on the application rates and timing of application is provided in **Table 1.**

5C. STUDY SITE LOCATION AND CHARACTERISTICS

Various aspects of the study sites are summarized in **Table 1.**

Table 1. Summary of cotton study site characteristics (treated sites only).

Attribute	Elko, South Carolina (Trial 1; BS001-18ZA)	Madera, California (Trial 2; BS003-18ZA)	Raymondville, Texas (Trial 3; BS007-18ZA)	Meridian, California (Trial 4; BS009-18ZA)
Variety	DP 348 RF Pima	DP-358 RF	Pima	Phytogen PHY 881 RF
Planting Date	May 4, 2018	July 30, 2018	March 20, 2018	May 15, 2018
Application Dates*	July 17 and 24, 2018	October 16 and 23, 2018	June 5 and 12, 2018	July 3 and 10, 2018
Air Temp (°F)	Not reported	Not reported	Not reported	Not reported
Humidity (%)	Not reported	Not reported	Not reported	Not reported
Timing*	BBCH 63 and BBCH 63	BBCH 61 and BBCH 63	BBCH 63 and BBCH 65	BBCH 61 and BBCH 63
Spray Volume (GPA)*	13 and 14	18 and 18	16 and 15	19 and 18
Rate (lbs ai/A)*	0.253 and 0.251	0.252 and 0.250	0.262 and 0.253	0.253 and 0.254
Adjuvant	None	None	None	None
Soil Type	Sand	Loamy sand	Sandy clay loam	Clay
OM (%)	0.5	0.7	1.8	2.0
pH	6.1	7.8	8.0	7.6
CEC (meq/100g)	3.1	7.4	33.4	34.6
Sand/Silt/Clay (%)	Not reported	Not reported	Not reported	Not reported

* The two values represent conditions during the first and second applications, respectively**.**

A summary of application, soil, and meteorological data from the three study sites is shown in **Table 1.** Trials were conducted on plots of sand (Trial 1), loamy sand (Trial 2), sandy clay loam (Trial 3) or clay soil (Trial 4). Soil organic matter varied from 0.5% to 2.0% for each site. The cotton crop was planted on May 4, 2018, July 30, 2018, March 20, 2018, and May 15, 2018 at Sites 1, 2, 3, and 4, respectively. The cotton test plots were grown and maintained according to typical local agricultural practices. Maintenance pesticides applied at the trial sites in the prior year included atrazine, s-metolachlor, glyphosate, aldicarb, diuron, and acetochlor at Trial 1; glyphosate, imidacloprid, and abamectin at Trial 2; atrazine, beta-cyfluthrin, terbufos, novaluron, glyphosate-isopropylammonium, zeta-cypermethrin, sulfoxaflor, tefluthrin, pendimethalin, spirotetramat, and chlorantraniliprole at Trial 3; and abamectin, diuron, thidiazuron, imidacloprid, glyphosate, and glufosinate-ammonium at Trial 4. Temperatures and rainfall during the field phase were similar to average historical records, with no significantly unusual weather conditions. Irrigation supplemented normal rainfall as needed. Rainfall occurred within 24 hours after the last test application at Site 1 (Elko, SC; 0.02 in).

5D. SAMPLE COLLECTION, HANDLING, PROCESSING

Cotton Plant Matrices and Sample Storage. Cotton flowers were collected by hand at 0, 2-3, and 6 days after the last application (DALA; BBCH 63-65) at all sites, while leaves were collected at 0 DALA only. Single composite samples of cotton flowers were harvested from the control plot for each trial within one

day of the corresponding sample from the treated plot. Composite flower samples contained a minimum of 500 flowers per subplot, collected from all areas of the cotton plant. Flowers were processed by hand on the day of sample collection to obtain extra-floral nectar, pollen, and floral nectar samples. Extrafloral nectar from the sub bracteal and inner bracteal nectaries were removed using a micropipette and placed into an amber vial. Pollen was removed from the cotton flowers by tapping the pollen from the flowers and collecting the accumulated pollen into an amber vial or into vacuum filter tips. Nectar from the floral nectary was removed by micropipette and placed in a separate amber vial. Composite leaf samples contained a minimum of 100 g.

All samples were stored frozen within four hours of collection and remained frozen until receipt at the analytical laboratory. Samples were stored frozen (<-18°C) at the analytical laboratory for a maximum of 219 days (7.2 months), prior to extraction.

5E. ANALYTICAL METHODS

Residues of spiromesifen and spiromesifen-enol were determined using high performance liquid chromatography/high resolution mass spectrometry (LC/HRMS) with stable isotopically labelled internal standards. To generate homogeneous samples, leaf samples were homogenized using a Robot Coupe chopper with dry ice. Details of the analytical methods are provided in the study report. The LOD/LOQ of spiromesifen and metabolite spiromesifen-enol in pollen, nectar, and leaves are shown in **Table 2**.

Table 2. Method LOD/LOQ in each matrix.

Analyte	Matrix	LOD (mg/kg)	LOQ (mg/kg)
Spiromesifen	Nectar	0.00021	0.0010
	Pollen	0.0012	0.010
	Leaves	0.0033	0.010
Spiromesifen-enol	Nectar	0.00011	0.0010
	Pollen	0.00068	0.010
	Leaves	0.0016	0.010

5F. QUALITY ASSURANCE

Transit Stability. Pollen and nectar samples were fortified at the analytical laboratory with spiromesifen and spiromesifen-enol at 0.010 ppm, shipped to the field sites, stored alongside test samples, and analyzed to determine stability of the analytes during transport and storage.

Freezer Stability. Freezer stability studies of spiromesifen and spiromesifen-enol were conducted separately.

Spike Recoveries. Concurrent recoveries were determined for spiromesifen and metabolite spiromesifen-enol in cotton leaves, nectar surrogate, and commercial pollen.

6. RESULTS:

6.A. QUALITY ASSURANCE RESULTS

Transit Stability. Mean recovery of spiromesifen from commercial pollen ranged from 70 to 76% (n = 2 per trial), and mean recovery of spiromesifen-enol ranged from 72 to 84% (n = 2 per trial) at a fortification of 0.10 mg/kg. Mean recovery of spiromesifen from surrogate nectar ranged from 81 to 88% (n = 2 per trial), and mean recovery of spiromesifen-enol ranged from 88 to 93% (n = 2 per trial) at a fortification of 0.10 mg/kg. Transit stability samples were analyzed following 7 months of storage.

Freezer Stability. It was stated that freezer storage stability studies conducted separately showed that spiromesifen and spiromesifen-enol are stable (<30% decomposition) during freezer storage for at least 11 months in the following representative crops: an oilseed (cotton undelinted seed); a nonoily grain (corn grain), a leafy vegetable (mustard green leaves), a root crop (potato tubers), and a fruit or fruiting vegetable (tomato whole fruit). Studies also demonstrated freezer stability for at least 11 months in the following raw and processed commodities: cotton gin trash, corn green forage and corn fodder, potato chips, flakes, and wet peel, and tomato paste and puree; and during freezer storage for at least 22 months in wheat forage, wheat hay, wheat grain, and turnip roots.

Spike Recoveries. All matrix spike mean recoveries from leaves, nectar, and pollen were within the acceptable range of 70 to 120% (**Table 3**).

Table 3. Concurrent Recoveries

Analyte	Matrix	Fortification Level (mg/kg)	Sample Size (n)	Mean (%)	RSD (%)
Spiromesifen	Leaves	0.10	1	96	-
	Nectar surrogate*	0.0010	8	98	7
		0.0050	6	96	7
		0.010	3	87	3
		0.10	8	96	2
		15	3	106	3
	Pollen**	0.010	7	79	5
		0.025	5	82	6
		0.10	11	79	10
Spiromesifen-enol	Leaves	0.10	1	102	-
	Nectar surrogate*	0.0010	8	97	4
		0.0050	6	98	5
		0.010	3	96	1
		0.10	8	95	2
		5.0	3	104	1
	Pollen**	0.010	7	89	2
		0.025	5	94	4
		0.10	11	91	4

* Commercial honey diluted with water to *ca*. 25% sugar content.

** Obtained from a local nutritional supplement store.

6.B. MAGNITUDE OF RESIDUES IN BEE-RELEVANT MATRICES

Cotton Pollen and Nectar. Summary statistics of the overall magnitude of total spiromesifen and the spiromesifen metabolites are shown in **Tables 4 through 17**. These statistics reflect analysis of individual composite samples among the sampling times. The parent spiromesifen accounted for the majority of total recovered residues in pollen at all sampling intervals at all four trials, and was typically higher in nectar samples. Spiromesifen was detected in pollen with maximum mean detections ranging from 0.7966 mg/kg at Trial 3 (Raymondville, Texas) to 6.9624 mg/kg at Trial 4 (Meridian, California), with the metabolite spiromesifen-enol maximum mean values ranging from 0.0481 mg/kg at Trial 3 (Raymondville, Texas) to 0.4157 mg/kg at Trial 2 (pooled sample; Madera, California). Mean concentrations of spiromesifen and total spiromesifen residues were higher in extrafloral nectar than floral nectar at all four trial sites. Spiromesifen was detected in extrafloral nectar at maximum means ranging from 0.3602 mg/kg at Trial 4 (Meridian, California) to 12.169 mg/kg at Trial 2 (Madera, California), with spiromesifen-enol maximum mean values ranging from 0.0219 mg/kg at Trial 4 (Meridian, California) to 2.809 mg/kg at Trial 2 (Madera, California). Spiromesifen and spiromesifen-enol were detected in floral nectar at maximum means 0.1234 mg/kg at Trial 4 (Meridian, California) and 0.0152 mg/kg at Trial 3 (Raymondville, Texas), respectively. The total spiromesifen residue value for each sample was calculated by summing the results for the two individual analytes (values below the LOD were assumed equal to ½ LOD, and values between the LOQ and LOD were assumed equal to ½ the LOQ).

Spiromesifen was detected in untreated pollen samples from Meridian, California at a maximum of 0.0368 ppm, while spiromesifen-enol was not quantifiable (<LOQ). Spiromesifen and spiromesifen-enol were both <LOD in untreated pollen samples from Elko, South Carolina and Raymondville, Texas.

Spiromesifen-enol was detected in untreated extrafloral nectar samples from Raymondville, Texas at up to 0.0017 ppm, and spiromesifen was not quantifiable (<LOQ). Spiromesifen and spiromesifen-enol were each detected between the LOD and LOQ in untreated extrafloral nectar samples from Meridian, California.

Table 4. Maximum analyte residues recovered from cotton pollen and nectar across all sampling dates.

Trial Site	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Pollen			
Elko, SC	8.2396	0.2143	8.4539
Madera, CA	4.6669*	0.4157*	5.0826*
Raymondville, TX	0.9101	0.0547	0.9648
Meridian, CA	7.8092	0.644	8.1693
Extrafloral Nectar			
Elko, SC	1.3441*	0.1927	1.3998*
Madera, CA	12.169*	2.809*	13.154*
Raymondville, TX	3.2457	1.004	4.2497
Meridian, CA	0.3602*	0.0219*	0.3821
Floral Nectar			
Elko, SC	0.0665	0.022	0.0709
Madera, CA	0.1137	0.0121	0.1258

Raymondville, TX	0.123	0.0191	0.1421
Meridian, CA	0.2451	0.0134	0.2521

* Pooled sample.

Table 5. Maximum mean analyte residues recovered from cotton pollen and nectar across all sampling dates.

Trial Site	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Pollen			
Elko, SC	5.2933	0.1224	5.4157
Madera, CA	4.6669*	0.4157*	5.0826*
Raymondville, TX	0.7966	0.0481	0.8437
Meridian, CA	6.9624	0.3427	7.2379
Extrafloral Nectar			
Elko, SC	1.3441*	0.1600	1.3998*
Madera, CA	12.169*	2.809*	13.154*
Raymondville, TX	2.8847	0.7499	3.6346
Meridian, CA	0.3602*	0.0219*	0.3821*
Floral Nectar			
Elko, SC	0.0486	0.0175	0.053
Madera, CA	0.0939	0.0101	0.1039
Raymondville, TX	0.0960	0.0152	0.1112
Meridian, CA	0.1234	0.0083	0.1311

* Pooled sample.

Table 6. Mean (min, max) concentrations of analytes in cotton pollen in Elko, South Carolina.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Pollen			
0	5.2933 (2.5994, 8.2396)	0.1224 (0.0751, 0.2143)	5.4157 (2.6745, 8.4539)
3	0.095 (0.0814, 0.1036)	0.0180 (0.0154, 0.0194)	0.1130 (0.0968, 0.1227)
6	0.0069 (<LOQ, 0.0107)	<LOQ (<LOQ, <LOQ)	0.0119 (0.010, 0.0157)

Table 7. Mean (min, max) concentrations of analytes in cotton pollen in Madera, California.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Pollen			
0	Not reported	Not reported	Not reported
3*	3.2416	0.1977	3.4393
6*	4.6669	0.4157	5.0826

* All samples were pooled for analysis.

Table 8. Mean (min, max) concentrations of analytes in cotton pollen in Raymondville, Texas.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)

Pollen			
0	0.7966 (0.6734, 0.9101)	0.0471 (0.0332, 0.0547)	0.8437 (0.70166, 0.9648)
2	0.6702 (0.562, 0.7412)	0.0481 (0.0426, 0.0514)	0.7183 (0.6046, 0.7916)
6	0.5255 (0.4824, 0.5622)	0.0379 (0.0362, 0.0405)	0.5635 (0.5229, 0.5984)

Table 9. Mean (min, max) concentrations of analytes in cotton pollen in Meridian, California.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Pollen			
0	6.9624 (6.1782, 7.8092)	0.2754 (0.2264, 0.3601)	7.2379 (6.4046, 8.1693)
2	2.9254 (0.6501, 6.6351)	0.2709 (0.049, 0.644)	3.1963 (0.6991, 7.2791)
6	2.8373 (2.0372, 3.3263)	0.3427 (0.2659, 0.3894)	3.1800 (2.3031, 3.7157)

Table 10. Mean (min, max) concentrations of analytes in cotton extrafloral nectar in Elko, South Carolina.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Extrafloral Nectar			
0*	1.3441	0.0557	1.3998
3**	0.1223 (0.0377, 0.2069)	0.1600 (0.1272, 0.1927)	0.2823 (0.2304, 0.3341)
6	0.0025 (0.0015, 0.0033)	0.0112 (0.0092, 0.0141)	0.0137 (0.0125, 0.0156)

* All samples were pooled for analysis.

** Samples from two subplots were pooled for analysis.

Table 11. Mean (min, max) concentrations of analytes in cotton extrafloral nectar in Madera, California.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Extrafloral Nectar			
0	Not reported	Not reported	Not reported
3*	12.169	0.985	13.154
6*	4.549	2.809	7.358

* All samples were pooled for analysis.

Table 12. Mean (min, max) concentrations of analytes in cotton extrafloral nectar in Raymondville, Texas.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Extrafloral Nectar			
0	2.3152 (1.5235, 3.1187)	0.3311 (0.2861, 0.3775)	2.6463 (1.8531, 3.4962)
2	2.8847 (2.5759, 3.2457)	0.7499 (0.5788, 1.004)	3.6346 (3.1547, 4.2497)
6	0.4260 (0.2143, 0.7332)	0.3432 (0.2985, 0.3722)	0.7693 (0.5865, 1.0317)

Table 13. Mean (min, max) concentrations of analytes in cotton extrafloral nectar in Meridian, California.

DALA	Spiromesifen	Spiromesifen-enol	Total

	(mg/kg)	(mg/kg)	(mg/kg)
Extrafloral Nectar			
0	Not reported	Not reported	Not reported
2*	0.3602	0.0219	0.3821
6	Not reported	Not reported	Not reported

* All samples were pooled for analysis.

Table 14. Mean (min, max) concentrations of analytes in cotton floral nectar in Elko, South Carolina.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Floral Nectar			
0	0.0486 (0.0359, 0.0665)	0.0044 (0.0043, 0.0044)	0.053 (0.0403, 0.0709)
3	0.0215 (0.0053, 0.0429)	0.0175 (0.0146, 0.022)	0.039 (0.0213, 0.0649)
6	<LOQ	0.0037 (0.0028, 0.0047)	0.0042 (0.0033, 0.0052)

Table 15. Mean (min, max) concentrations of analytes in cotton floral nectar in Madera, California.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Floral Nectar			
0	0.0939 (0.0823, 0.1137)	0.0101 (0.0077, 0.0121)	0.1039 (0.0927, 0.1258)
3	0.0692 (0.041, 0.1052)	0.0076 (0.007, 0.008)	0.0768 (0.048, 0.113)
6*	0.0183 (0.0082, 0.0284)	0.0036 (0.0033, 0.0038)	0.0219 (0.0015, 0.0322)

* Only two replicates available.

Table 16. Mean (min, max) concentrations of analytes in cotton floral nectar in Raymondville, Texas.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Floral Nectar			
0	0.0960 (0.0543, 0.123)	0.0152 (0.0096, 0.0191)	0.1112 (0.0639, 0.1421)
2	0.0643 (0.0377, 0.1037)	0.01 (0.0078, 0.0127)	0.0743 (0.0472, 0.1164)
6	0.0104 (0.0075, 0.0148)	0.0085 (0.0062, 0.0103)	0.0189 (0.0165, 0.021)

Table 17. Mean (min, max) concentrations of analytes in cotton floral nectar in Meridian, California.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Floral Nectar			
0	0.1234 (0.0578, 0.2451)	0.0077 (0.007, 0.0092)	0.1311 (0.0648, 0.2521)
2	0.0467 (0.0334, 0.0595)	0.0066 (0.0057, 0.0076)	0.0532 (0.041, 0.0652)
6	0.0173 (0.0051, 0.0333)	0.0083 (0.0044, 0.0134)	0.0256 (0.0095, 0.0467)

Trends in spiromesifen and total spiromesifen residue concentrations declined in pollen samples from 0 DALA to 6 DALA at all trial sites excluding Trial 2 (Madera, California), where maximum pooled residues were observed at 6 DALA (**Figures 1-4**). Spiromesifen and total spiromesifen residues declined in extrafloral nectar samples from Trials 1-3 following maximum mean concentrations detected at 0-3 DALA

and from floral nectar samples following 0 DALA (insufficient extrafloral nectar samples were collected at Trial 4 to determine a trend; **Figures 5-11**).

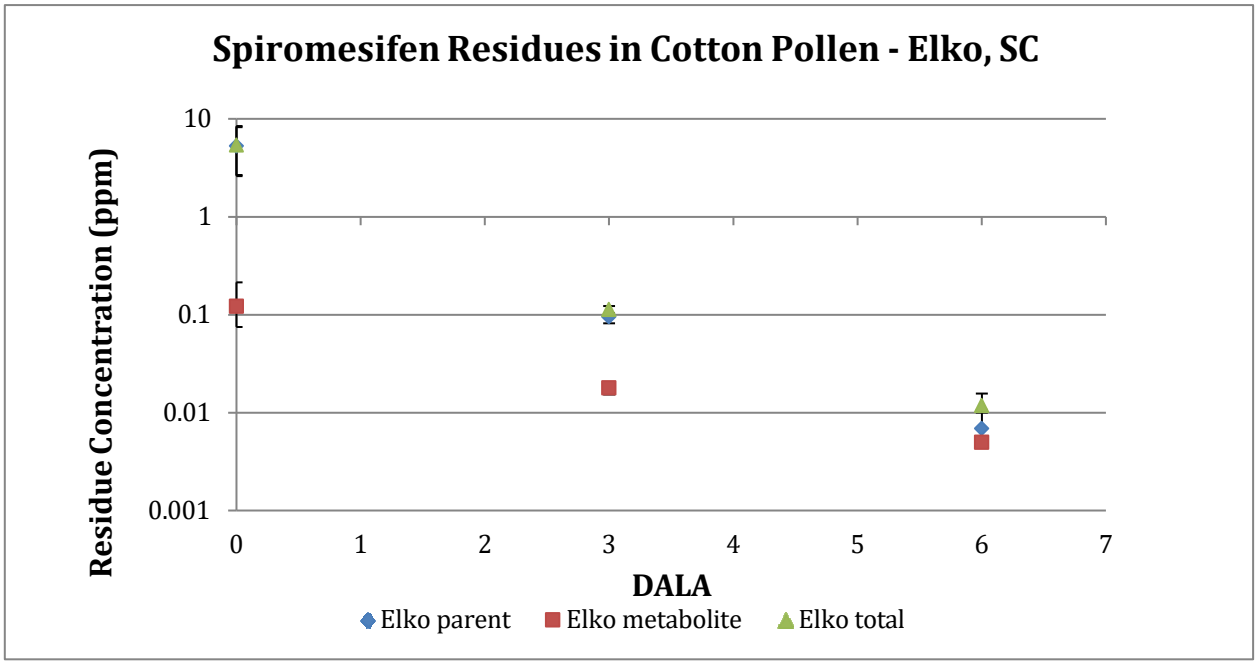

Figure 1. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton pollen at Elko, South Carolina. Error bars represent maximum and minimum replicate values.

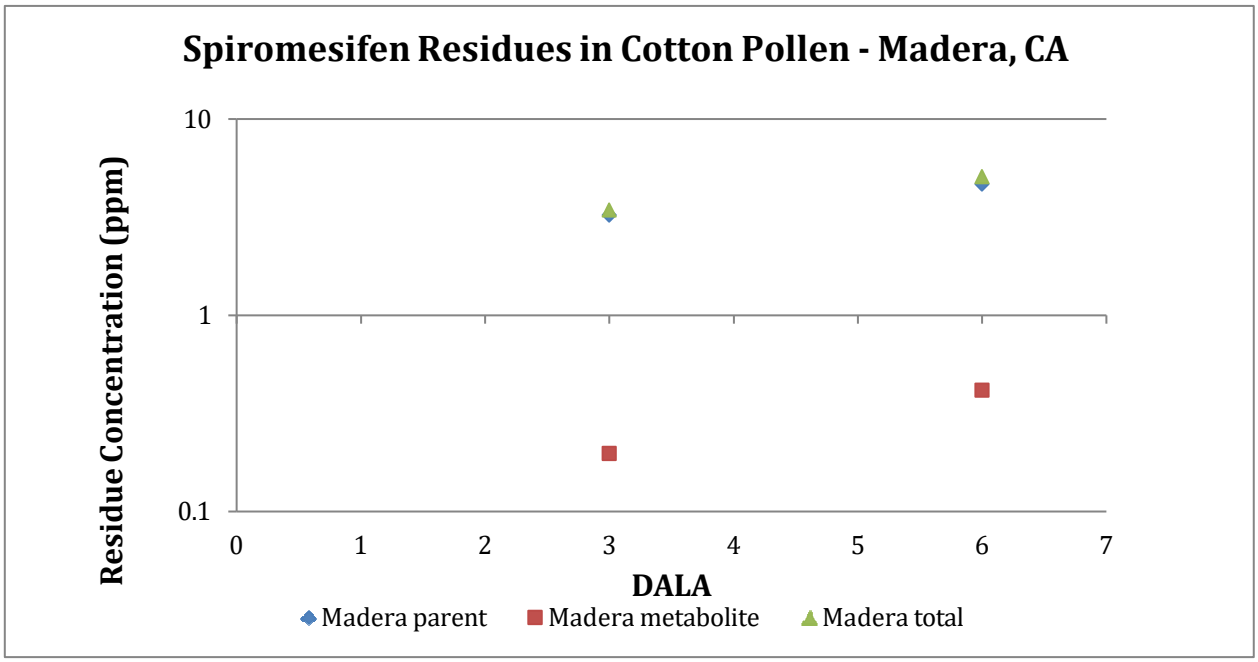

Figure 2. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton pollen at Madera, California. Error bars represent maximum and minimum replicate values.

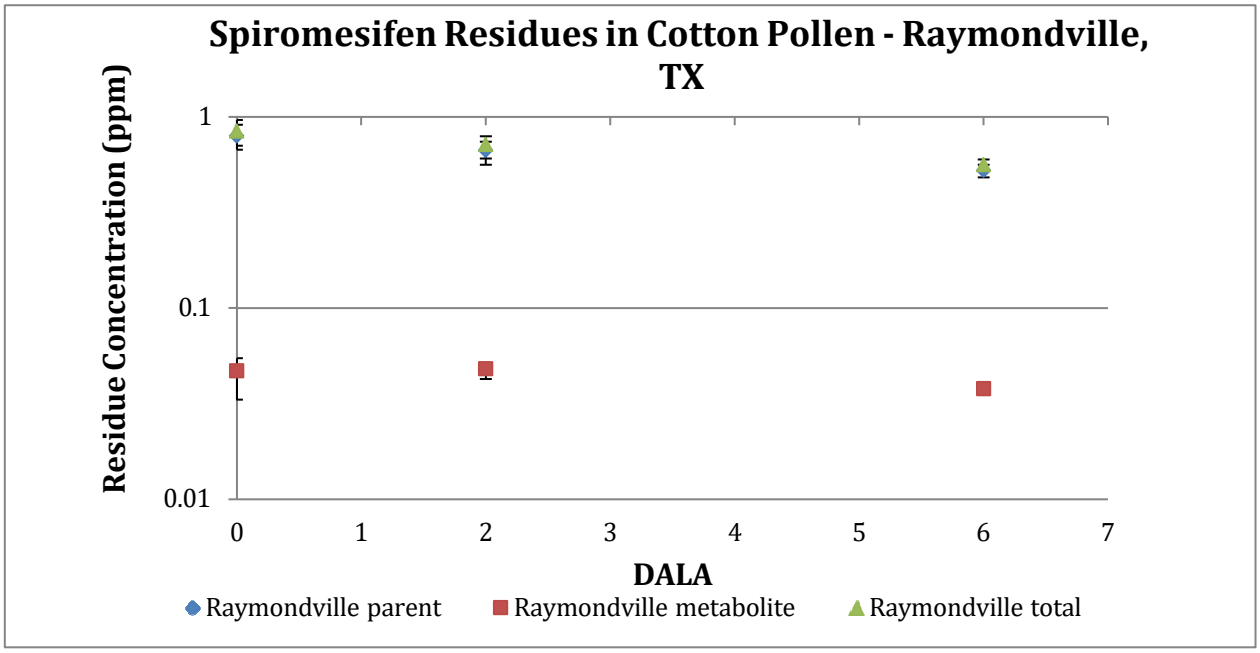

Figure 3. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton pollen at Raymondville, Texas. Error bars represent maximum and minimum replicate values.

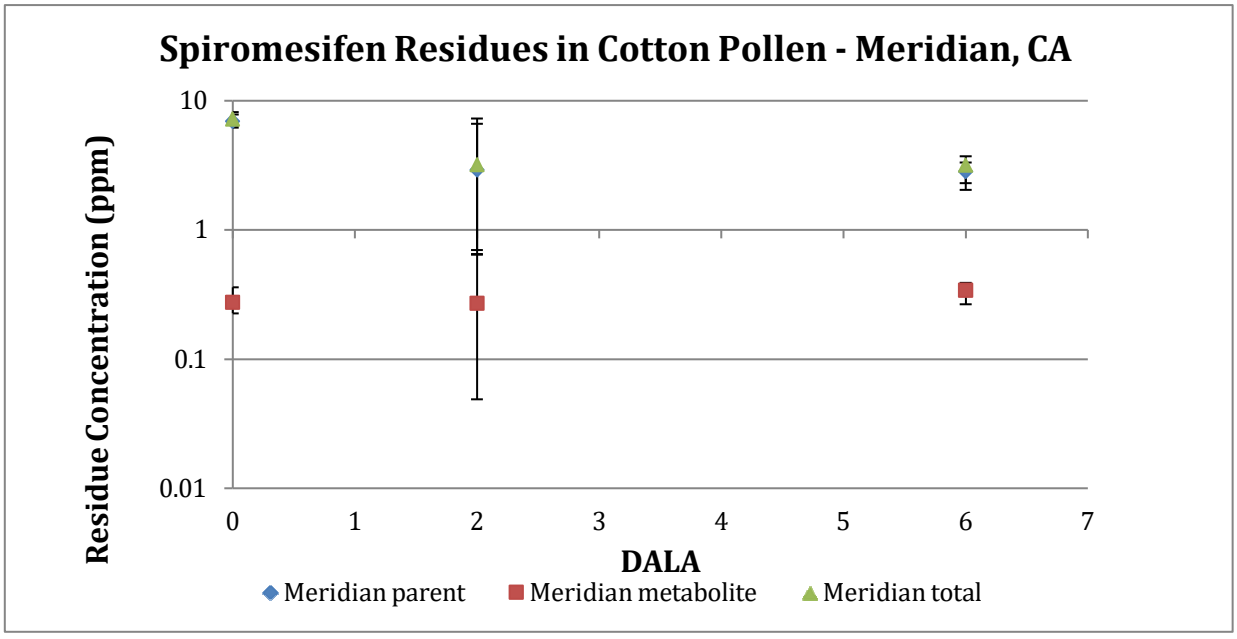

Figure 4. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton pollen at Meridian, California. Error bars represent maximum and minimum replicate values.

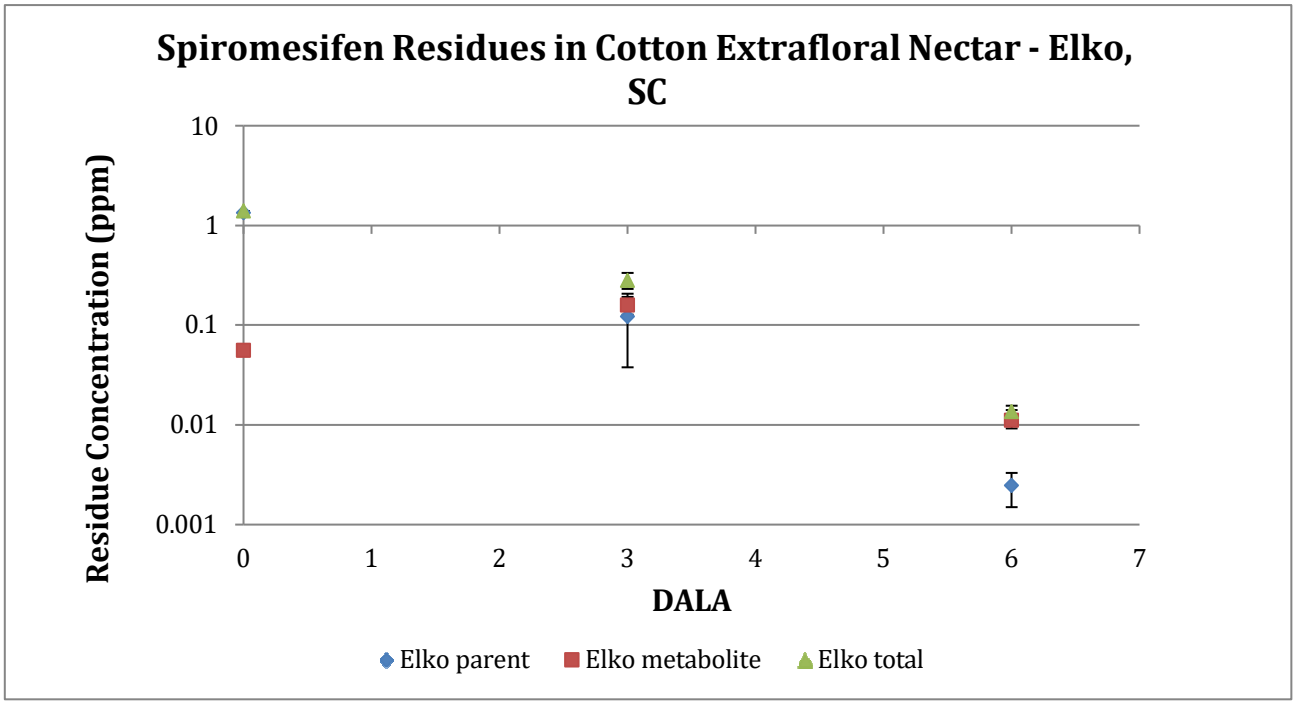

Figure 5. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton extrafloral nectar at Elko, South Carolina. Error bars represent maximum and minimum replicate values.

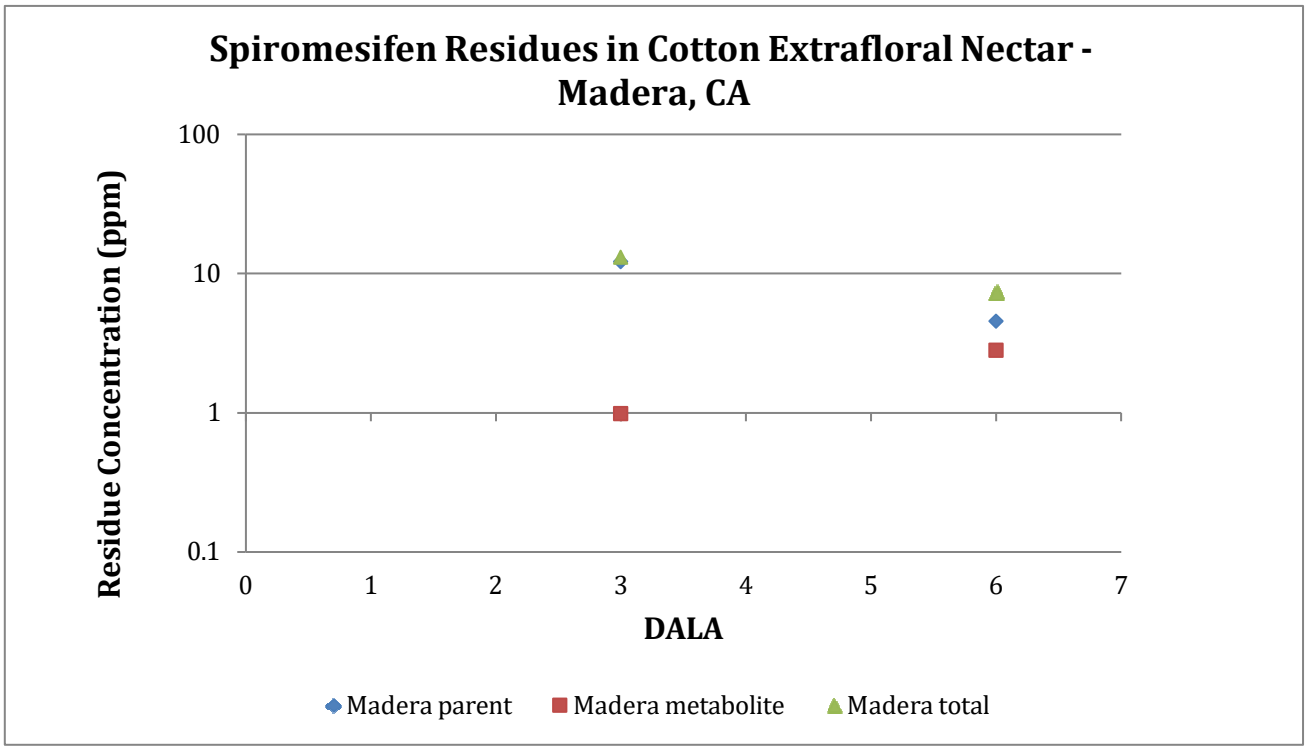

Figure 6. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton extrafloral nectar at Madera, California. Error bars represent maximum and minimum replicate values.

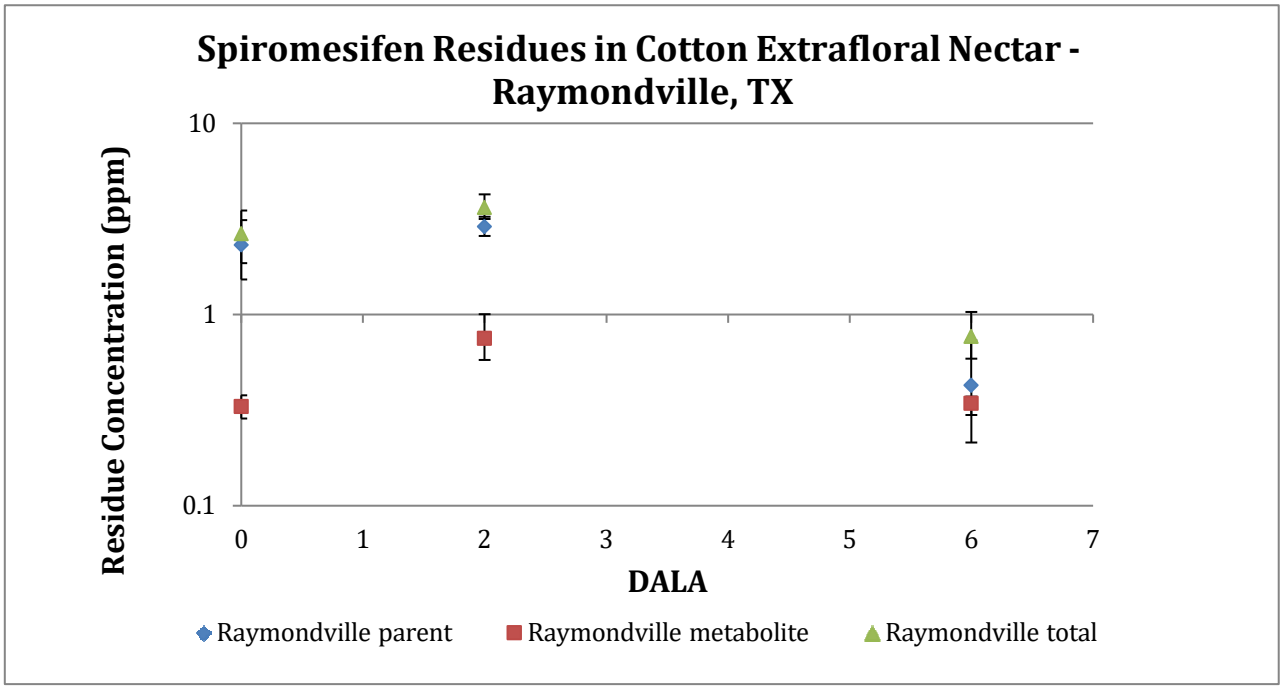

Figure 7. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton extrafloral nectar at Raymondville, Texas. Error bars represent maximum and minimum replicate values.

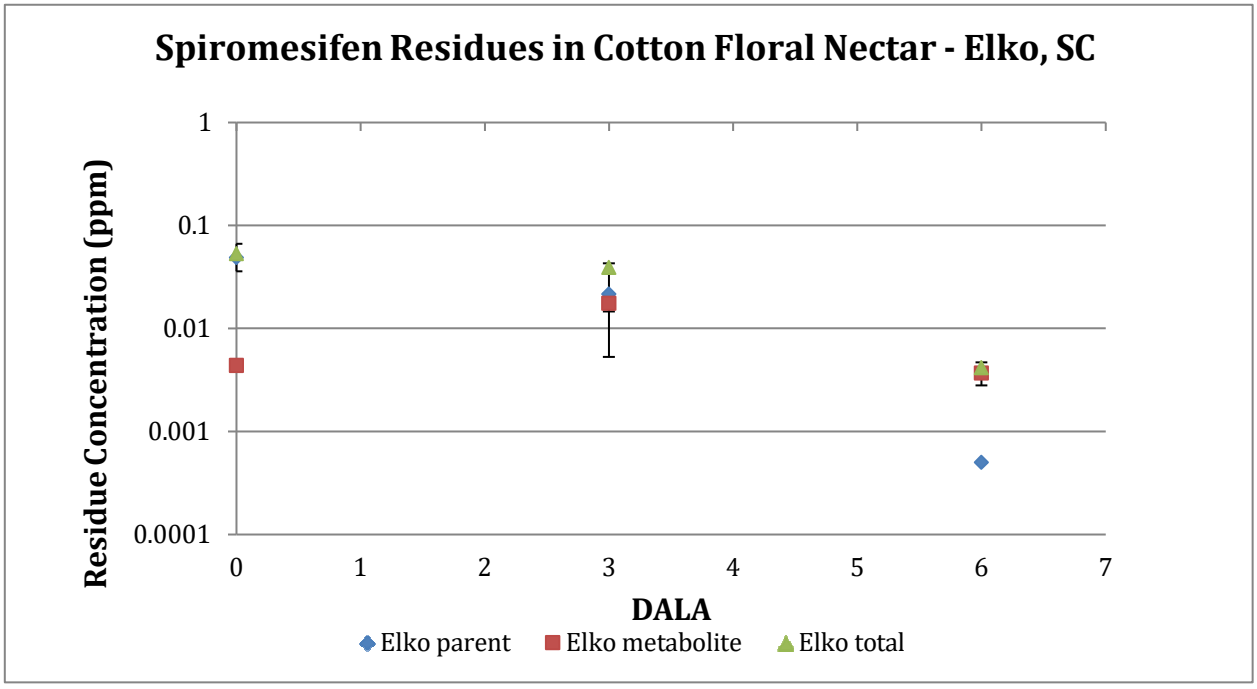

Figure 8. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton floral nectar at Elko, South Carolina. Error bars represent maximum and minimum replicate values.

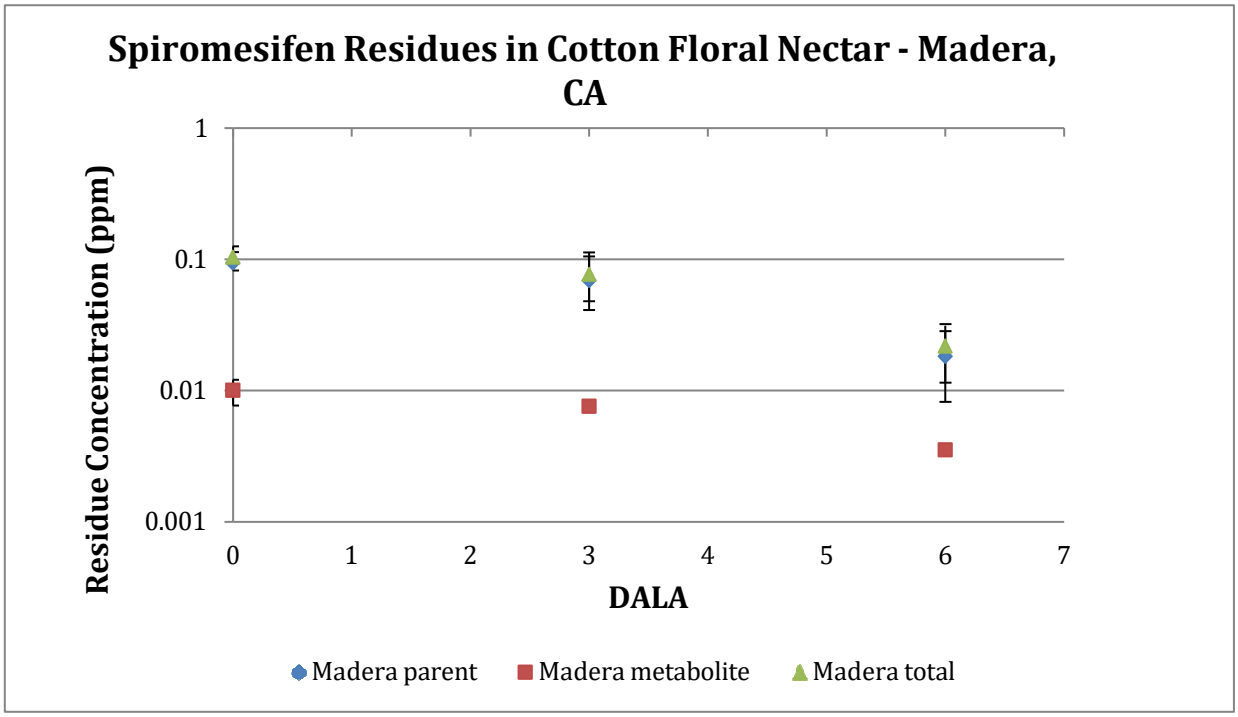

Figure 9. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton floral nectar at Madera, California. Error bars represent maximum and minimum replicate values.

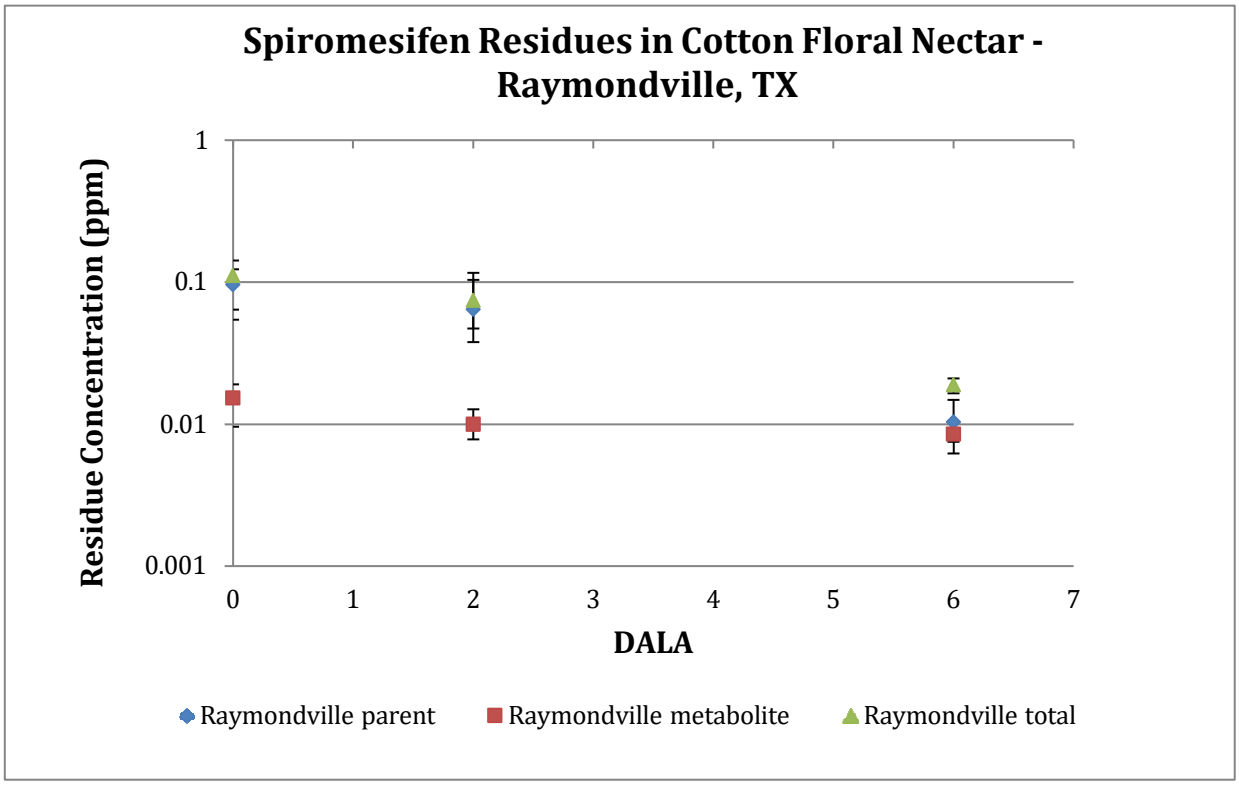

Figure 10. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton floral nectar at Raymondville, Texas. Error bars represent maximum and minimum replicate values.

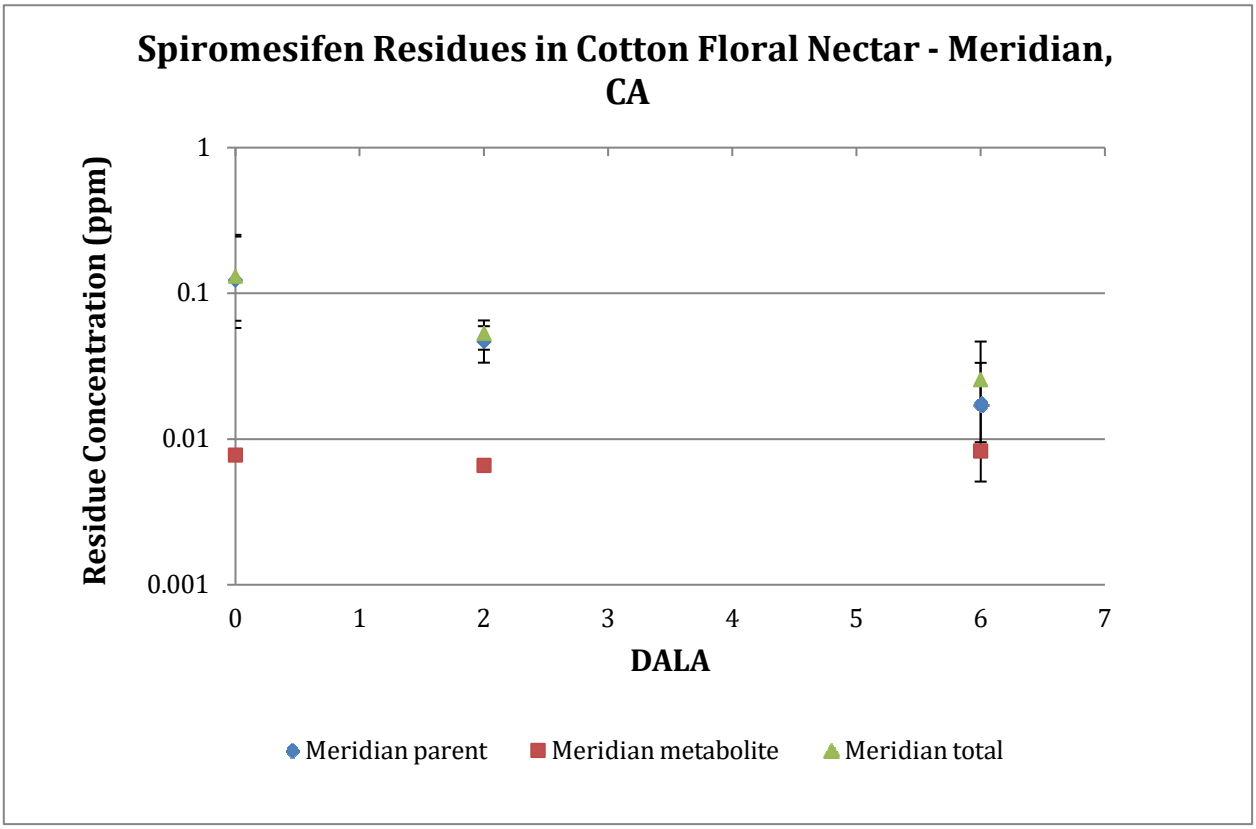

Figure 11. Mean measured spiromesifen, spiromesifen-enol, and total spiromesifen residues in cotton floral nectar at Meridian, California. Error bars represent maximum and minimum replicate values.

6.C. MAGNITUDE OF RESIDUES IN LEAVES

Summary statistics of the overall magnitude of total spiromesifen and the spiromesifen metabolites are shown in **Tables 18** to **21**. Parent spiromesifen accounted for the majority of total recovered residues in leaves at the 0 DALA sampling interval at all three trials. Spiromesifen was detected in leaves with maximum mean detections ranging from 40.61 mg/kg at Trial 3 (Raymondville, Texas) to 54.53 mg/kg at Trial 4 (Meridian, California), with spiromesifen-enol maximum mean values ranging from 0.75 mg/kg at Trial 2 (Madera, California) to 1.41 mg/kg at Trial 4 (Meridian, California). The total spiromesifen residue value for each sample was calculated by summing the results for the two individual analytes.

Spiromesifen was detected in untreated leaf samples from Madera, California at 0.0106 ppm. Spiromesifen and spiromesifen-enol were not detected in any other leaf samples or trials.

Table 18. Mean (min, max) concentrations of analytes in cotton leaves in Elko, South Carolina.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Leaves			
0	42.3520 (35.8258, 47.7478)	1.2366 (1.1622, 1.281)	43.5887 (36.988, 49.0288)

Table 19. Mean (min, max) concentrations of analytes in cotton leaves in Madera, CA.

DALA	Spiromesifen	Spiromesifen-enol	Total

	(mg/kg)	(mg/kg)	(mg/kg)
Leaves			
0	53.1611 (47.9531, 59.9463)	0.7531 (0.6782, 0.8756)	53.9143 (48.6313, 60.8219)

Table 20. Mean (min, max) concentrations of analytes in cotton leaves in Raymondville, TX.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Leaves			
0	40.6135 (37.3779, 44.4365)	1.2505 (1.0178, 1.378)	41.864 (38.3957, 45.7922)

Table 21. Mean (min, max) concentrations of analytes in cotton leaves in Meridian, CA.

DALA	Spiromesifen (mg/kg)	Spiromesifen-enol (mg/kg)	Total (mg/kg)
Leaves			
0	54.5344 (45.5326, 61.8906)	1.4139 (1.0421, 1.7978)	55.9483 (46.5747, 63.6884)

Trends in spiromesifen residue concentrations following two foliar applications in leaves could not be determined.

6.D. RESIDUE DECLINE (DT_{50}) IN COTTON MATRICES

Pollen and nectar samples were only collected three times and leaves only collected one time, from each treatment area, thereby preventing the determination of DT_{50} values. No analyses were conducted.

7. STUDY STRENGTHS, LIMITATIONS AND CONCLUSIONS

In the context of documenting the magnitude of spiromesifen residues in bee-related matrices of cotton resulting from foliar application, the following strengths are observed with this study.

1. Concentrations were measured for toxicologically-relevant metabolites in multiple plant matrices.
2. Application methods and rates were well documented.
3. Sampling contained a reasonable amount of replication and compositing to account for natural variability.
4. Trials were conducted across four different locations in three different regions of the country (EPA Region 2, South Carolina; EPA Region 10, California; and EPA Region 6, Texas), with varying soil types. This allowed for comparison of residue magnitudes and trends in cotton matrices across varying climatic conditions and soil types, while holding plant species constant.
5. The two applications were the seasonal maximum number of foliar applications permitted according to the label to reflect commercial worst-case scenarios.
6. Analytical methods were generally sufficiently accurate and precise based on QA data.

The following limitations were noted with this study:

1. Residues were only measured over a single growing season, thereby preventing the determination of carry over and year to year variability.
2. Pollen and nectar samples were only collected three times from each treatment area (leaves one

time), thereby preventing the determination of DT_{50} values.
3. Soil samples were not collected, and leaves were only collected at 0 DALA.
4. Concentrations of the parent material and/or metabolite were detected >LOQ in control plots from Madera, CA, Raymondville, TX, and Meridian, CA, indicating the potential for field contamination.
5. Data were not reported for certain pollen and nectar sampling intervals across the trial sites. The field data summary reports indicated that samples were collected. However, it was not clear why the samples were not analyzed.

Overall, considering the strengths and limitations of this study, the following conclusions can be drawn:

1. Two foliar applications of Oberon 2® SC to cotton plants at BBCH 61-63 at a nominal application rate of 0.25-0.26 lb ai/A, yields detectable residues of spiromesifen in pollen and nectar throughout the 0-6 DALA study period at all trial sites, and leaves at 0 DALA.
2. In cotton matrices, spiromesifen residues were greatest in leaves (mean maximums of 40.61-54.53 mg/kg for each trial site), followed by extrafloral nectar (mean maximums of 0.36-12.17 mg/kg for each trial site), pollen (mean maximums of 0.8-6.96 mg/kg for each trial site), and floral nectar (mean maximums of 0.05-0.12 mg/kg for each trial site). The parent material accounted for the majority of total recovered residues in leaves, nectar, and pollen. Spiromesifen-enol residues were greatest in extrafloral nectar, followed by leaves, pollen, and floral nectar (mean maximums ranged from 0.02-2.8 mg/kg for each trial site).
3. Trends in spiromesifen and total spiromesifen residue concentrations following two foliar applications declined in pollen samples from 0 DALA to 6 DALA at all trial sites excluding Trial 2 (Madera, California), where maximum pooled residues were observed at 6 DALA. Spiromesifen and total spiromesifen residues declined in extrafloral nectar samples from Trials 1-3 following maximum mean concentrations detected at 0-3 DALA and from floral nectar samples following 0 DALA (insufficient extrafloral nectar samples were collected at Trial 4 to determine a trend).
4. DT50 values of spiromesifen, spiromesifen-enol, and total spiromesifen residues could not be calculated in nectar, pollen, or leaves at all trial sites due to an insufficient number of sampling intervals.

8. STUDY VALIDITY/CLASSIFICATION

Data from the four study locations are considered scientifically sound and useful for risk assessment purposes, although these trials were conducted within a single growing season. Overall, this study is classified as **ACCEPTABLE** for quantitative use in risk assessment.